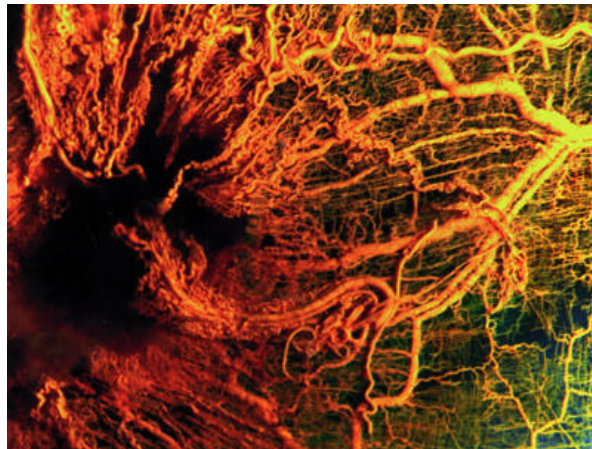


ECOG Pharmacogenomics: Anti-Angiogenic Agents

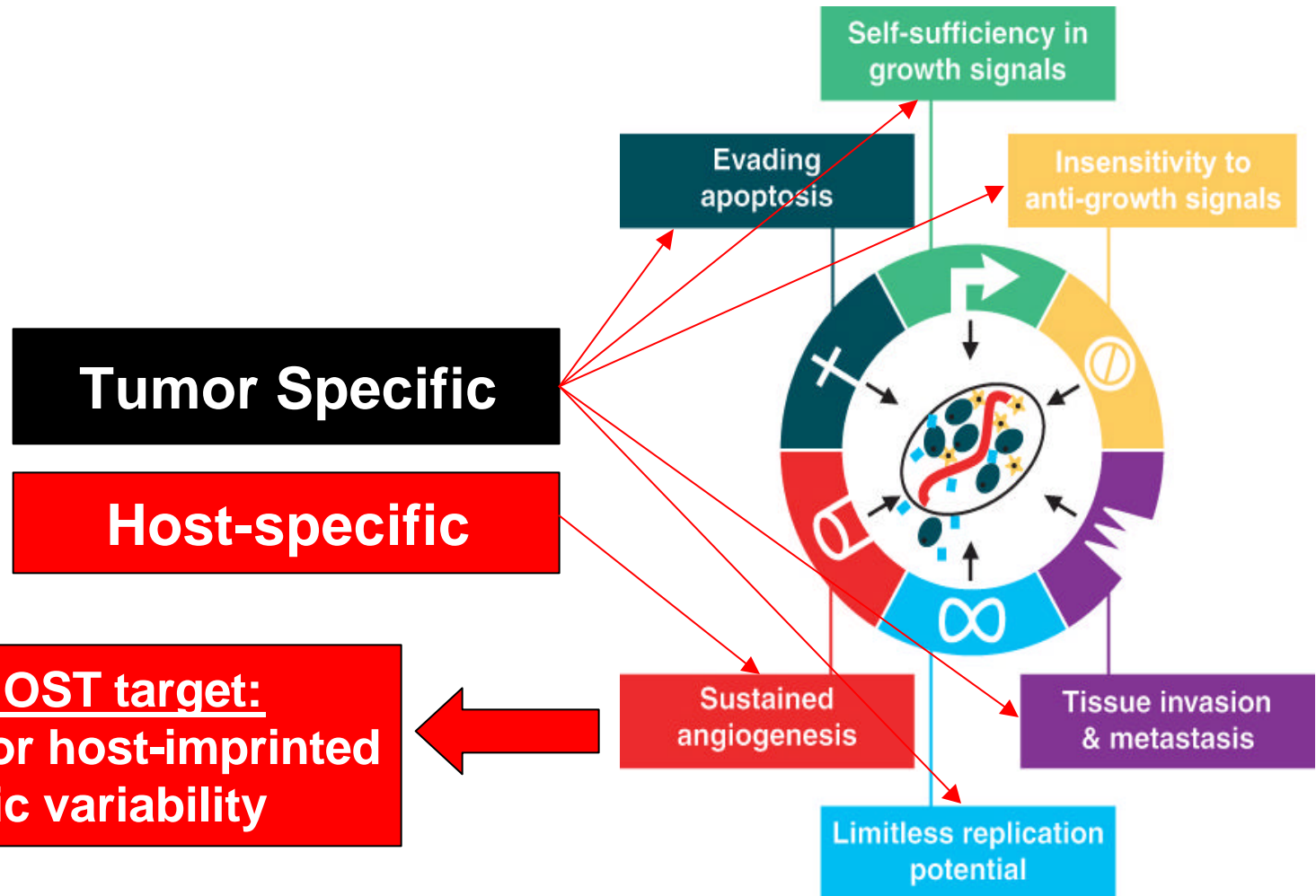


Bryan P. Schneider, MD

George Sledge MD, Kathy Miller MD, David Flockhart MD, PhD,
Todd Skaar PhD, Sunil Badve PhD.

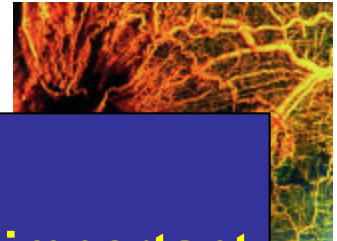
COBRA, Indiana University

Hallmarks of malignancy: a biomarker rich environment?



Genetic variability impacts angiogenesis: brief summary

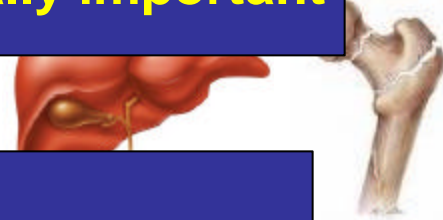
- Epidemiologic data:
 - **Variable risk & prognosis in multiple conditions where angiogenesis is important:** risk/prognosis in multiple malignancies, retinopathy, nephropathy, pre-eclampsia,



NOT Level 1 evidence

Body of data: strongly suggests variability is biologically important

- Variability may associate with site of metastasis

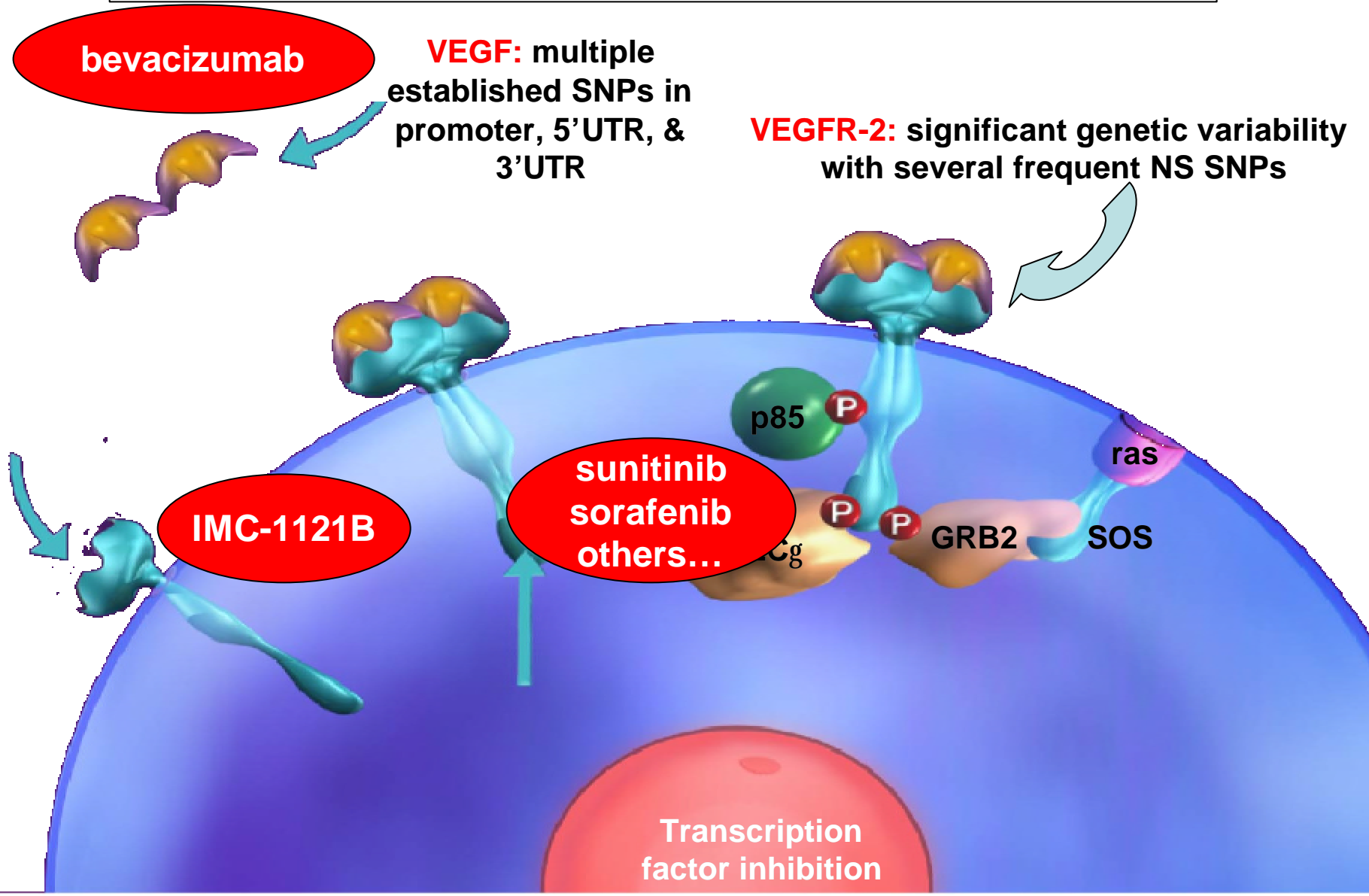


Breast cancer angiogenesis as a model

- variability in complement factor H may affect treatment outcome in macular degeneration (?biomarker)
 - CC genotype had inferior outcome in visual acuity with intravitreal bevacizumab



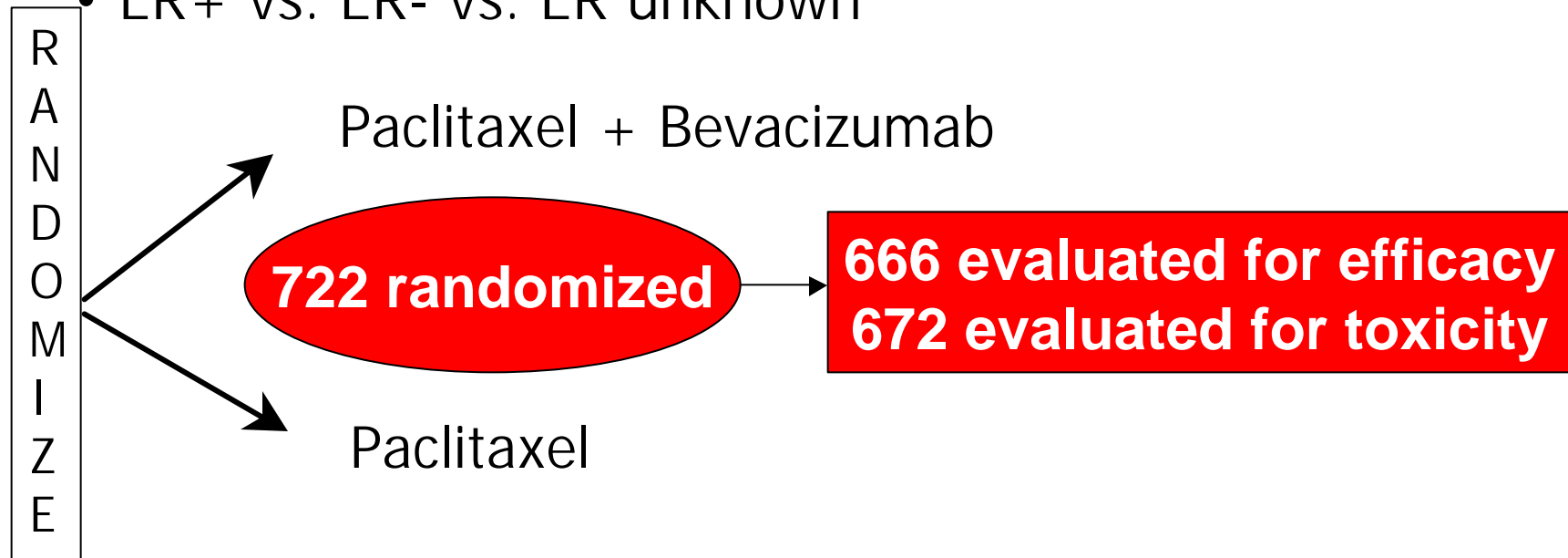
Excellent genetic variability in angiogenesis drug targets



Bevacizumab in breast cancer-E2100: a model of therapeutic heterogeneity

Stratify:

- DFI \leq 24 mos. vs. $>$ 24 mos.
- < 3 vs. ≥ 3 metastatic sites
- Adjuvant chemotherapy yes vs. no
- ER+ vs. ER- vs. ER unknown



Bevacizumab increased grade 3/4 toxicity

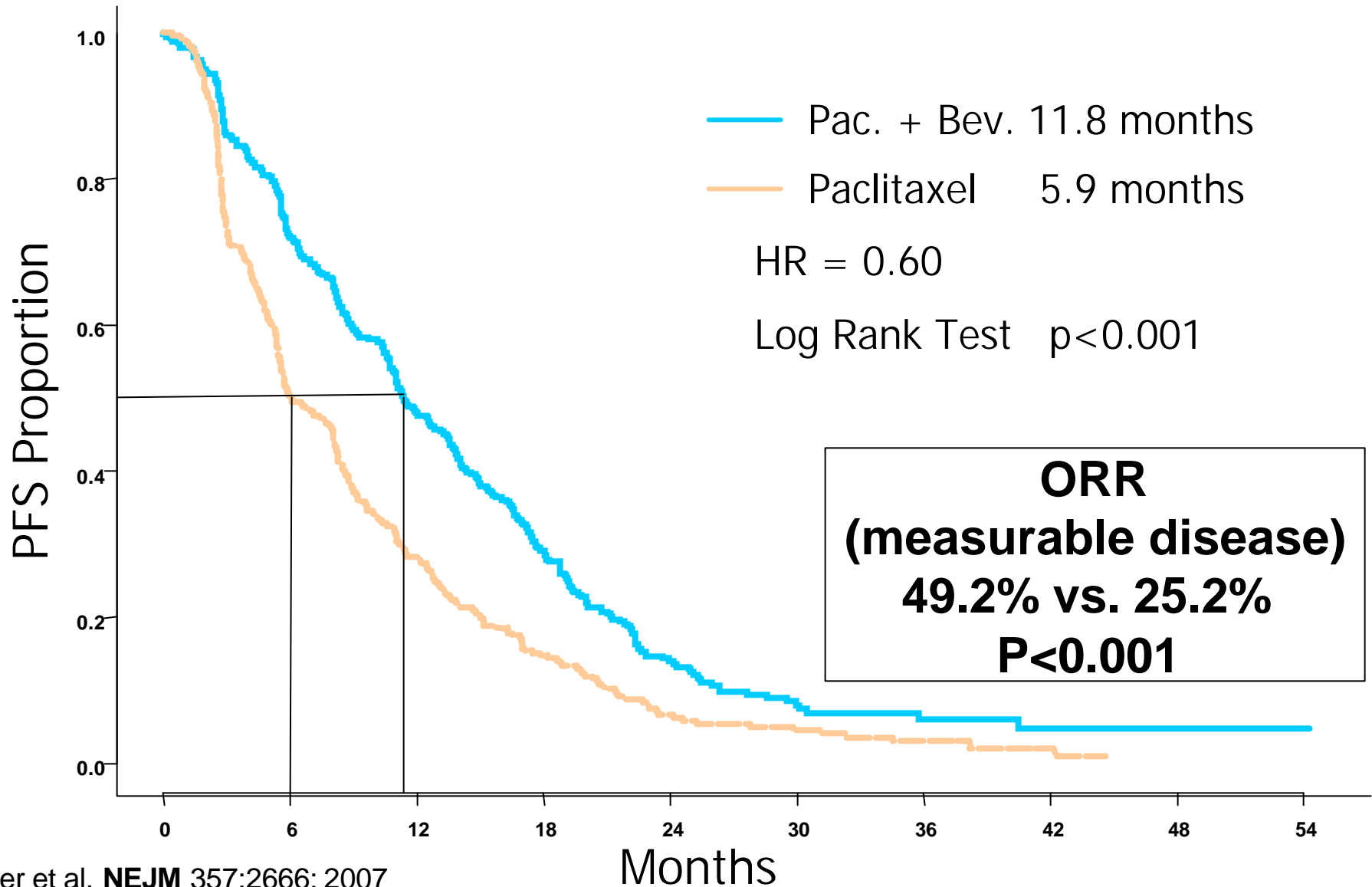
Serious, frequent, & unique

Serious but rare

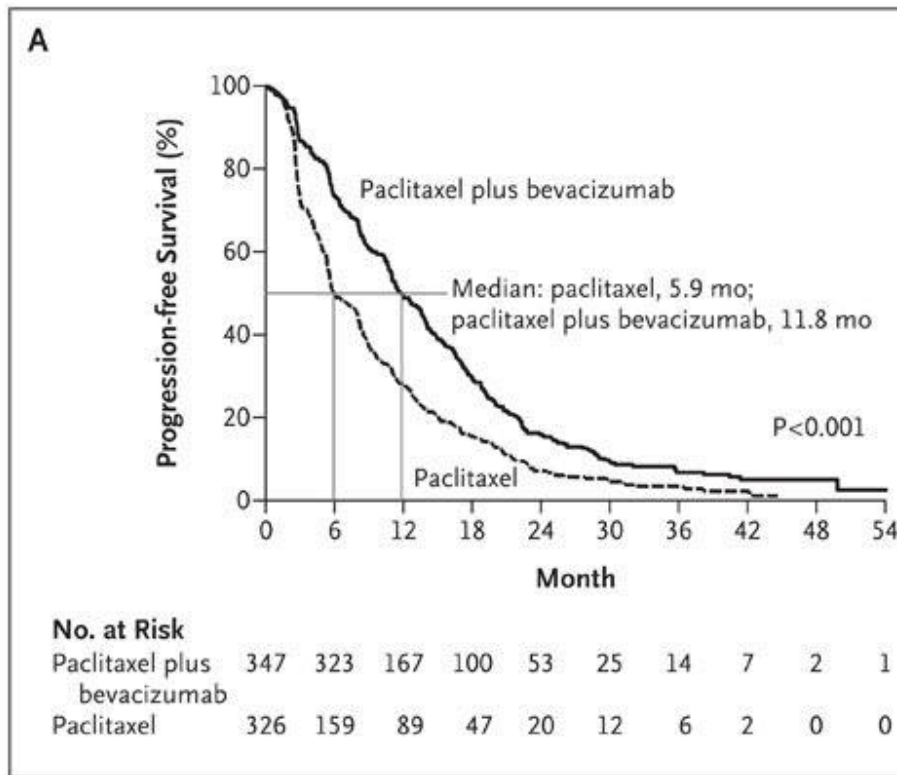
Likely related to duration of taxane exposure

Toxicity	P (%)	P+B (%)	p-value
Infection	2.9	9.3	<0.001
Fatigue	4.9	9.1	0.04
Neuropathy	17.7	23.5	0.05
CNS ischemia	0	1.9	0.02
Headache	0	2.2	0.008
Proteinuria	0	3.5	<0.001
Hypertension	0	14.8%	<0.001

Bevacizumab significantly improved PFS



Improvement in PFS/ORR did not translate into OS benefit



ORR
(measurable disease)
49.2% vs. 25.2%
P<0.001



Attempts to find surrogate markers for response to bevacizumab unsuccessful to date

- Tumor VEGF, Thrombospondin-2, k-ras, k-raf, p53 & MVD did **NOT** correlate with survival for patients with metastatic colon cancer treated with bevacizumab
- Baseline serum VCAM & urine VEGF did **NOT** correlate with outcome in E2100

Jubb et al., **JCO**, 24, 217-227; 2006

Ince et al., **J Natl Cancer Inst**, 97:981-9, 2005

Miller et al. **SABCS**, Abstract#3: 2005

Germline genetic variation in tumor angiogenesis

- This an excellent place to study role of germline genetic variation!
 - Hallmark of malignancy
 - Other active drugs against angiogenesis
 - Balanced heterogeneity
 - Clear benefit vs. no benefit
 - Frequent, unique, non-overlapping toxicities
 - “Targeted therapy” without a population to target
 - Tumor angiogenesis is genetically diverse
 - Variation appears to been inherited (vs. mutations)

E2100 Pharmacogenomics: a TBCI-catalyzed study

- Evaluate for correlation between VEGF/VEGFR-2 SNPs (*from primary tumor*) & efficacy
 - PFS (primary endpoint)
 - Overall Survival
 - OR
- Evaluate for correlation between VEGF/VEGFR-2 SNPs (*from primary tumor*) & toxicity
 - Clinically significant hypertension (Grade 3/4)
- Evaluate for association between SNPs & expression (IHC)
- Evaluate for association between expression (IHC) & outcomes

Candidate SNPs meet fundamental requirements

- **Biologic rationale:**
 - Impact on breast ca risk/other
 - Reasonable likelihood will alter gene function and/or production
- **Genes are clear drug targets:**
 - VEGF/VEGFR-2
- **High frequency of rare allele:**
 - VEGF SNPs: 15-49%
 - VEGFR-2 SNPs: 9-25%

Candidate SNPs:

VEGF

-2578 C/A, -1498 C/T,
-1154 G/A, -634 G/C,
& +936 C/T

VEGFR-2

V297I, & Q472H

E2100 Pharmacogenomics: a TBCI-catalyzed study

- Evaluate for correlation between VEGF/VEGFR-2 SNPs (*from primary tumor*) & efficacy
 - PFS
 - Overall Survival
 - ORR
- Evaluate for correlation between VEGF/VEGFR-2 SNPs & expression (IHC)
- Evaluate for association between expression (IHC) & outcomes

Why did we not use germline DNA?

Can we assume polymorphic sites evaluated are same in tumor and host?

Genetic variability in tumor angiogenesis is identical to germline DNA

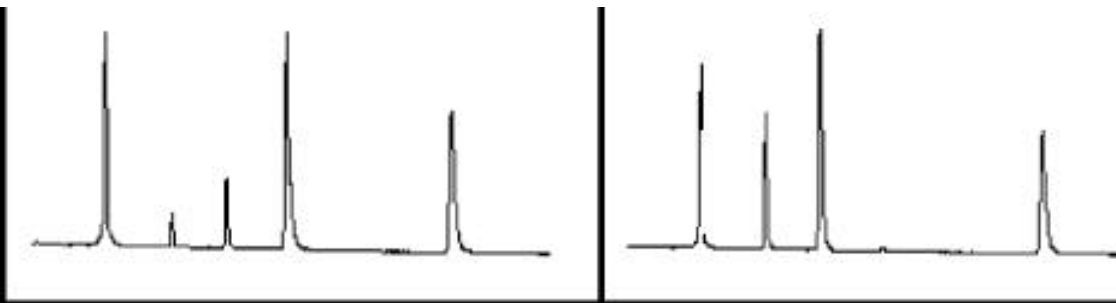


Table 3. Genotypic results of polymorphism of VEGF

Case #	Primary tumor	Lymph node +	Lymph node -
#1	w/w	w/w	
#2		w/w	w/w
#3	w/ var	w/ var	w/ var
#4	w/w	w/w	w/w
#5			var / var
#6	w/w		
#7			w/w
#8			w/w
#9	0	0	w/ var
#10	w/w	w/w	w/w
#11	w/ var	w/ var	w/ var
#12	w/ var	w/ var	W/ var
#13	w/w	w/w	w/w
#14	w/w	w/w	w/w
#15	w/w	w/w	w/w
#16	w/w	w/w	w/w
#17	0	w/ var	w/ var
#18	W/w	w/w	w/w
#19	w/ var	w/ var	w/ var
#20	w/ var	w/ var	w/ var
#21	w/w	w/w	W/w

Genotypic results for the $C^{936}T$ polymorphism of the VEGF gene for all 21 cases. Blank spaces indicate no sample submitted for that case number/site.

- **Genotype-** (21 women with breast cancer)
 - Primary breast tumor (n=17)
 - LN+ (n=17) & LN- (n=19)
 - VEGF 936 C/T
 - eNOS Promoter (-786 T/C) & Exon 7 E298D
- All polymorphisms (combined sites)
 - high quality chromatographs in 145 of 159 (91%)
 - 100% concordance** between samples that involved malignancy (primary or LN+) vs germline (95% CI, 0.88 to 1.00)

E2100 correlative study:

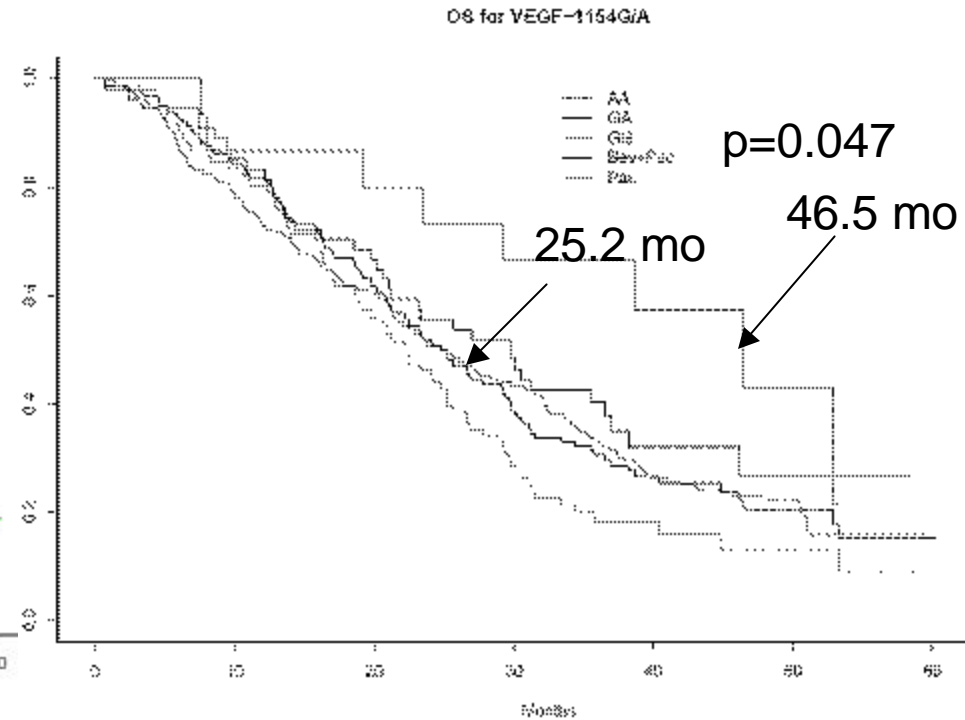
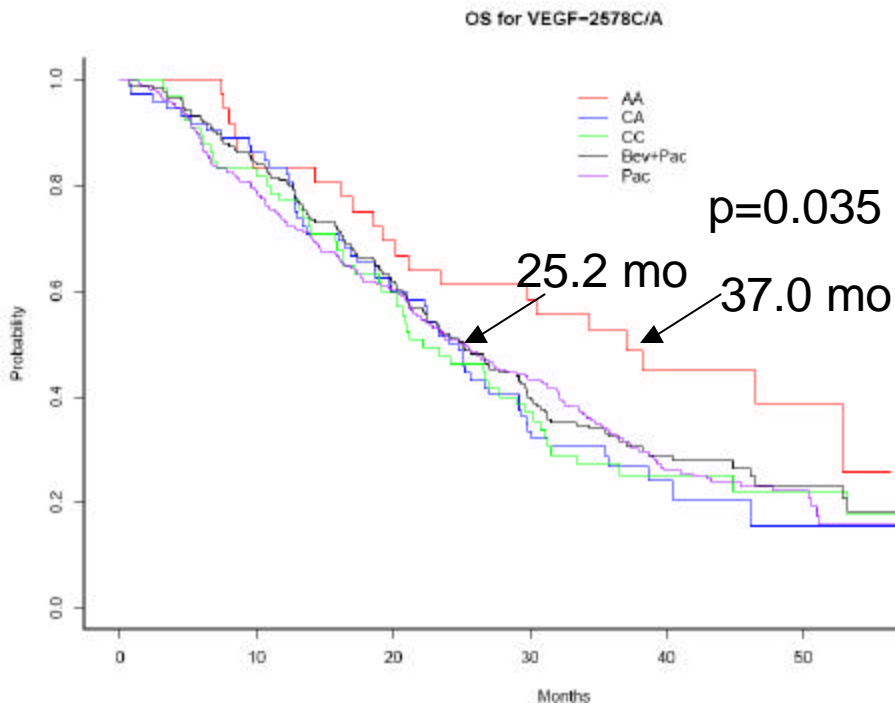
Methods

- 673 eligible pts & 623 dz progression (11/07)
- DNA extracted from paraffin embedded tumor blocks (genotype-363; VEGF IHC-377; VEGFR2-341)
 - ~50% from experimental arm
- Genotyping of candidate SNPs (Real time-PCR)
 - **VEGF**: -2578 C/A, -1498 C/T, -1154 G/A, -634 G/C, 936C/T
 - **VEGFR-2**: V297I & Q472H
- IHC for VEGF & VEGFR-2 tumor expression

VEGF -2578 AA & -1154 AA genotypes associated with improved OS in combination arm

SNP	Genotype comparison (median OS in mo & freq)	HR	CI	P-value
VEGF-2578	CA (24.4; 42.6%) vs. AA (37.0; 20.8%)	1.78	(98.3%=0.96, 3.32)	0.026
	CC (22.2; 37.6%) vs. AA (37.0; 21%)	1.70	(98.3%=0.91, 3.17)	0.043
	CC (22.2; 37.6%) vs. CA (24.4; 42.6%)	0.99	(98.3%=0.62, 1.58)	0.95
	AA vs. CA+CC	0.58	(95%=-0.36, 0.93)	0.023
VEGF-1154	GG (22.3; 56.9%) vs. GA (29.8; 38.8%)	1.60	(98.3%=0.98, 2.60)	0.022
	GG (22.3; 56.95) vs. AA (46.5; 9.4%)	2.69	(98.3%=1.10, 6.59)	0.008
	GA (29.8; 38.8%) vs. AA (46.5; 9.4%)	1.68	(98.3%=0.66, 4.30)	0.19
	AA vs. GA vs. GG	0.62	(95%=0.46, 0.83)	0.001

VEGF -2578 AA & -1154 AA genotypes in combination arm outperformed control



Median OS

Control arm=25.2 mo
Combination arm=26.7 mo
Combination arm AA=37.0 mo

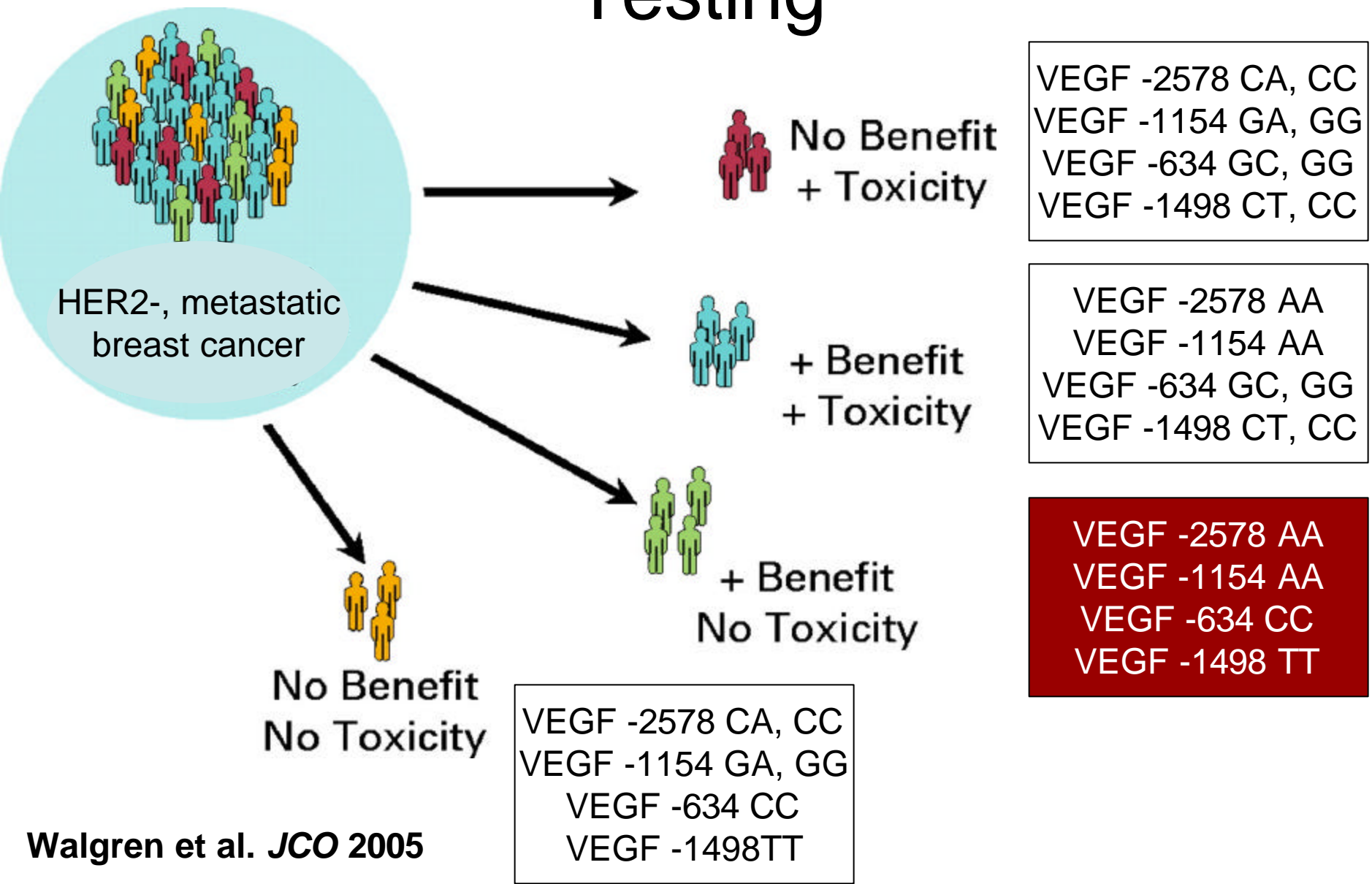
Median OS

Control arm=25.2 mo
Combination arm=26.7 mo
Combination arm AA=46.5 mo

Genetic variability of VEGF predicts clinically significant hypertension in E2100

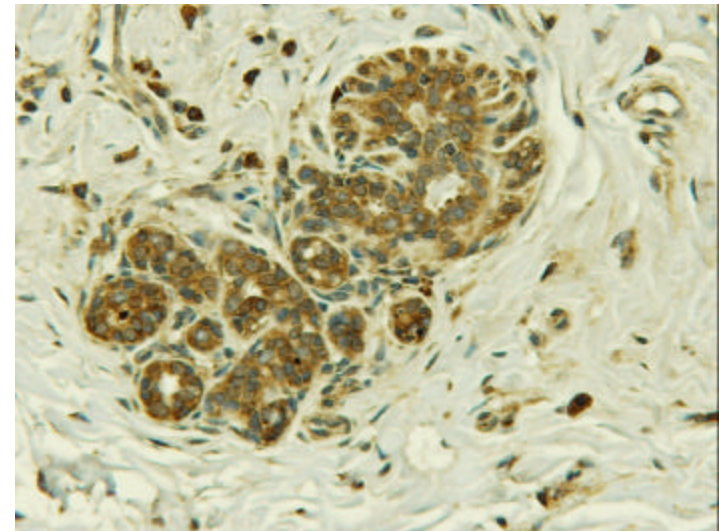
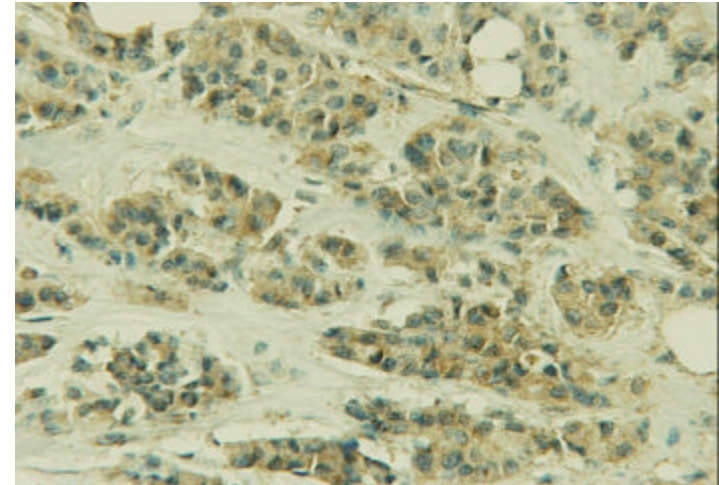
SNP	% Grade 3/4 hypertension (#/%) by genotype	p-value
VEGF-634	CC=0% (n=27; 15.3%) vs. GC=22% (n=82; 46.3%) vs. GG=19% (n=68; 38.4%)	0.013
	CC vs. GC+GG	0.005
VEGF-1498	TT=8% (n=60; 33.9%) vs. CT=22% (n=82; 46.3%) vs. CC=23% (n=35; 19.8%)	0.056
	TT vs. CC+CT	0.022

The Promise of Pharmacogenetic Testing



VEGF genotypes may be associated with tumor expression

- VEGF genotype trended toward a correlation with VEGF expression
 - Prior preclinical data suggest VEGF **-2578A** & **-1154A** alleles have lower expression
 - **VEGF-2578 AA** genotype had lower VEGF expression ($p=0.12$) vs. alternate genotypes
 - **VEGF-1154 AA** genotype had lower VEGF expression ($p=0.08$) vs. alternate genotypes
 - Does this provide some sort of mechanistic clue??
 - Host-mediated changes in plasma VEGF after angiogenesis therapy-(*Ebos, Kerbel PNAS 2007*)
- VEGF & VEGFR-2 expression did not correlate with outcome in E2100



What are the mechanistic explanations for our clinical findings??

- Background/Rationale
 - Data suggest there is a role for variability in outcome **BUT:**
 - SNPs & haplotypes not fully defined (PGRN/NHLBI Sequencing in Process)
 - Prior pre-clinical promoter studies are incomplete

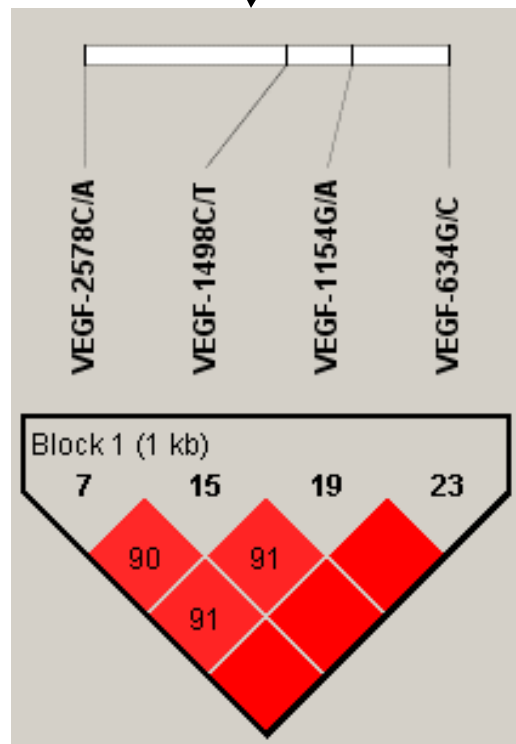
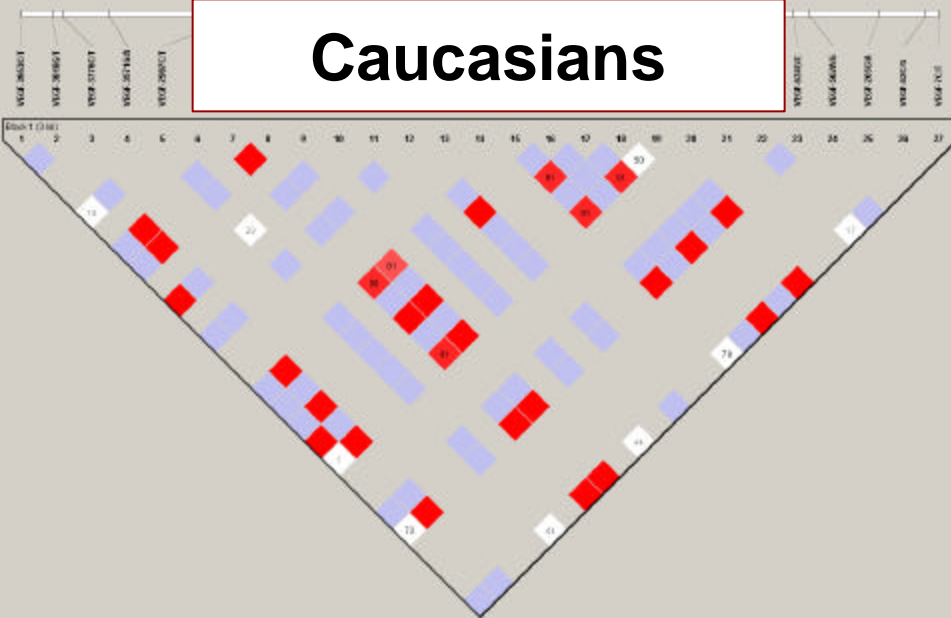


- Plan
 - Re-sequence promoter & 5'-UTR
 - Definitively establish genetic variation & haplotypes
 - Evaluate role of SNPs on promoter activity

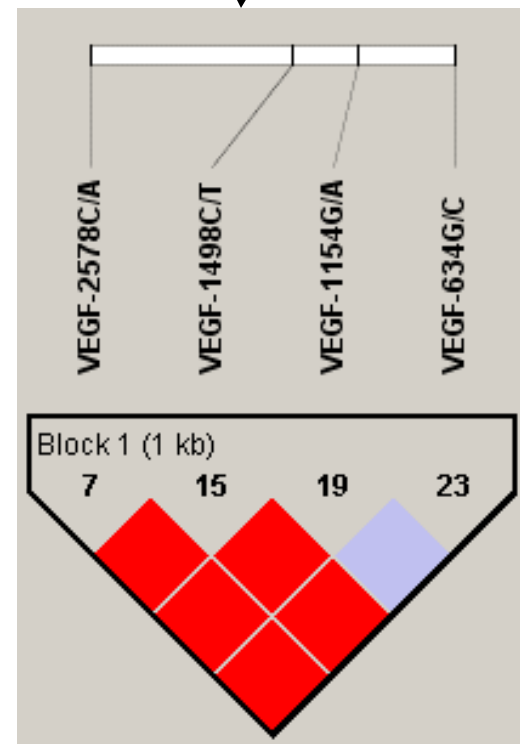
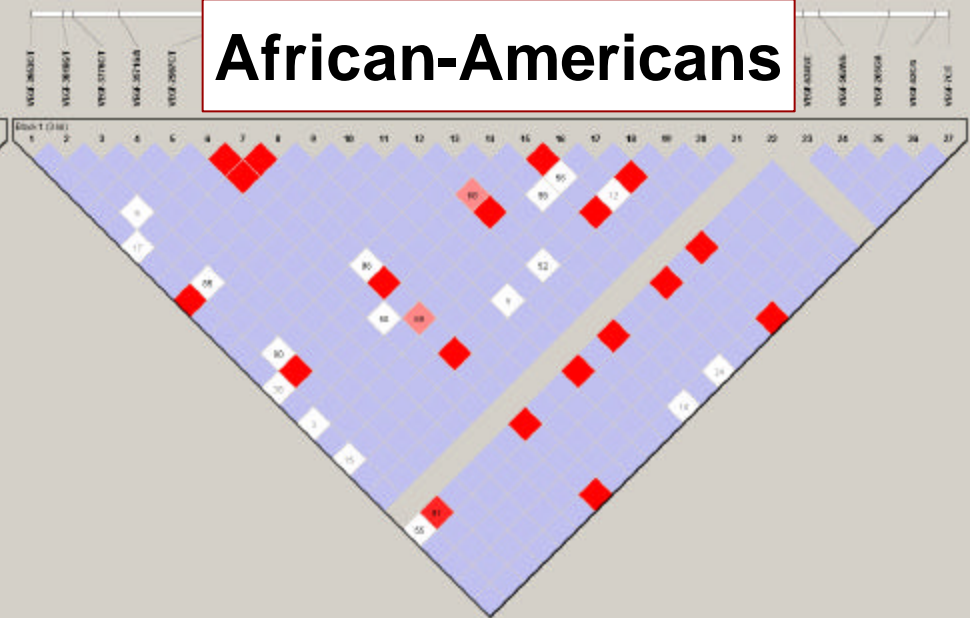
VEGF Promoter & 5'-UTR Re-sequencing

- 4.0kb upstream of “ATG” start codon
 - 96 samples from Coriell Repository
 - 48 Caucasians, 48 African Americans
 - Captures all known transcription factor binding sites
 - Contains a high density of SNPs
- Identified 19 SNPs
 - 16 of 19 SNPs previously reported (NCBI)
 - 13 common & 3 rare (<5% frequency)
 - 5 of 13 common had no prior population frequency
 - 3 of 19 SNPs are novel (not previously reported)
 - 1 common & 2 rare
- Currently cloning VEGF promoter variants into expression vectors for luciferase studies

Caucasians



African-Americans



Caucasians

01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	
C	G	C	G	C	T	A	T	G	G	C	C	G	C	C	C	A	A	A	C	G	G	G	A	G	C	C	.323
C	T	C	G	C	T	C	C	G	G	C	C	G	C	T	C	G	A	G	C	G	G	C	A	G	C	C	.198
C	G	C	G	C	T	A	T	G	G	C	C	G	C	C	C	A	A	G	C	G	G	G	A	G	C	T	.166
C	G	C	G	C	T	C	C	G	G	C	C	G	C	T	C	G	A	G	C	G	G	G	A	G	C	C	.073
x	G	C	G	C	T	C	C	G	G	C	C	G	C	T	C	G	x	G	C	G	G	G	A	G	C	C	.052
C	G	C	G	C	T	A	T	G	G	C	C	G	C	T	C	A	A	(
C	G	C	G	C	T	C	C	G	G	C	C	G	C	T	C	G	A	(
x	G	C	G	C	T	C	C	G	x	C	C	G	C	T	C	G	A	(
C	G	C	G	x	T	C	C	G	G	C	C	G	C	T	C	G	A	(
C	G	C	G	C	T	C	C	G	G	C	C	G	C	T	C	G	A	(
C	G	C	G	C	T	C	C	G	G	C	C	G	C	C	C	G	A	(
C	G	C	G	C	T	A	T	G	G	C	C	G	C	C	C	A	A	(
C	G	C	G	C	T	C	C	G	G	C	x	G	C	C	x	G	A	(
x	G	C	G	C	T	C	C	G	G	C	C	G	C	T	C	G	A	/									

76%

African-Americans

01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27			
C	T	C	G	C	T	C	C	G	G	C	C	G	C	T	C	G	A	G	C	G	G	C	A	G	C	C	.202		
C	G	C	G	C	T	C	C	G	G	C	C	G	C	T	C	G	A	G	C	G	G	G	A	G	C	C	.138		
T	G	C	G	C	T	C	C	G	x	C	C	G	C	T	C	G	A	G	C	G	G	C	A	G	C	C	.128		
C	G	C	G	C	T	C	C	G	G	C	A	G	C	C	A	G	A	G	C	G	G	G	A	G	C	C	.085		
T	G	C	G	C	T	C	C	G	G	C	C	G	C	T	C	A	A	G	C	G	G	G	A	G	C	C	.053		
C	C	C	C	C	T	A	C	C	C	C	C	C	C	C	C	C	C	C	A	A	x	C	G	G	G	A	G	C	.037
C	C	A	A	x	C	G	G	G	A	G	C	C															.037		
T	C	G	A	G	C	G	G	G	A	G	C	C															.032		
T	C	G	A	G	C	G	G	G	A	G	C	C															.032		
C	C	A	A	G	C	G	G	G	A	G	C	x															.027		
C	C	A	A	G	C	G	G	G	A	G	C	x															.027		
T	C	G	A	G	C	G	G	G	A	G	C	C															.021		
C	C	G	A	G	C	G	G	G	A	G	C	C															.021		

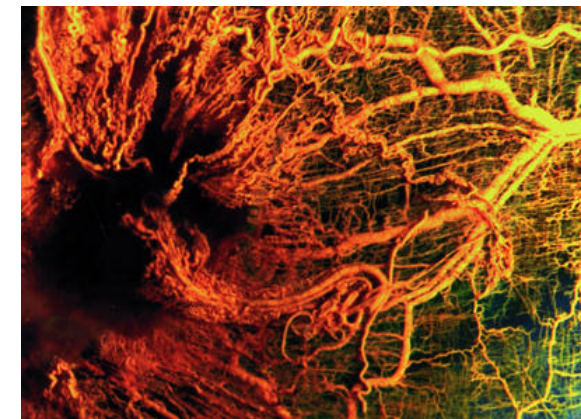
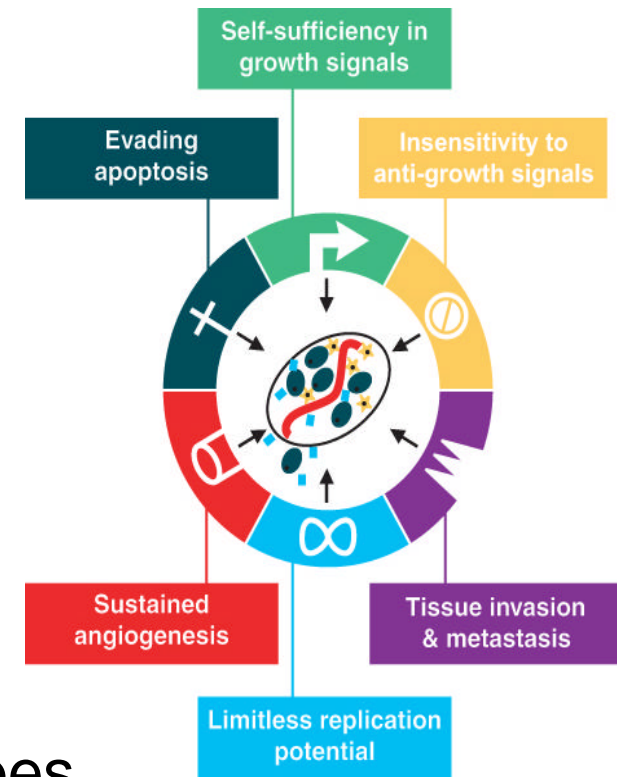
01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	
C	G	C	G	C	T	A	T	G	G	C	C	G	C	C	C	A	A	A	C	G	G	G	A	G	C	C	.205
C	T	C	G	C	T	C	C	G	G	C	C	G	C	T	C	G	A	G	C	G	G	C	A	G	C	C	.200
C	G	C	G	C	T	A	T	G	G	C	C	G	C	C	C	A	A	G	C	G	G	G	A	G	C	T	.110
C	G	C	G	C	T	C	C	G	G	C	C	G	C	T	C	G	A	G	C	G	G	G	A	G	C	C	.103
T	G	C	G	C	T	C	C	G	x	C	C	G	C	T	C	G	A	G	C	G	G	C	A	G	C	C	.079
C	G	C	G	C	T	C	C	G	G	C	A	G	C	C	A	G	A	G	C	G	G	G	A	G	C	C	.042
T	G	C	G	C	T	C	C	G	G	C	C	G	C	T	C	G	x	G	C	G	G	G	A	G	C	C	.032
C	G	C	G	T	T	C	C	G	G	C	C	G	C	T	C	G	A	G	C	G	G	G	A	G	C	C	.026
C	G	C	G	C	T	A	T	G	G	C	C	G	C	C	C	A	A	G	C	G	G	G	A	G	C	C	.021
T	G	C	G	C	T	C	C	G	G	C	C	G	C	T	C	A	A	G	C	G	G	G	A	G	C	C	.021
C	G	C	G	C	T	C	C	G	G	C	C	G	C	T	C	G	A	G	C	G	G	C	A	G	C	C	.017

70%

47%

Conclusions

- Pharmacogenetics (biomarkers)
 - Improves therapeutic index
 - Leads to drug discovery
 - Benefits patients
- Angiogenesis
 - Hallmark of malignancy
 - Inhibition effective in multiple tumor types
 - Therapeutic heterogeneity→biomarkers needed
 - Early work suggests germline genetic variability might be important
 - Validation and further understanding of molecular biology essential



“Host Hallmark”

Acknowledgements

- David Flockhart MD, PhD
- Milan Radovich
- Bradley Hancock
- Jason Robarge
- Lang Li, PhD
- Faouzi Azzouz,
- Suzanne Lemler, RN
- Todd Skaar, PhD
- Anne Nguyen
- Sunil Badve, MD
- George Sledge, MD
- Kathy Miller, MD
- Anna Maria Storniolo, MD
- Connie Rufenbarger
- “Friends for Life Coalition”

Supported by: ASCO Career Development Award, BCRF, GCRC CreFF Award, Catherine Peachey Fund, & IU Simon Cancer Center

“VEGF low” expressers (-2578AA, -1154AA)

